Dear Readers

Finally, the third and last issue of special supplements on the state of the art and perspectives of alternative methods to animal experimentation now appears thereby rounding up the first two issues published in winter 2001/02 and fall 2003.

The motivations for performing animal experiments are manifold. While pharmaceutical companies attempt to develop new and efficient products as quickly and economically as possible, controlling agencies such as the FDA are interested in highly predictive testing systems that keep the many risks of substances under control. On the other hand, research scientists are mostly driven by their personal curiosity and professional interest to break new ground and to contribute new bouts of knowledge that might be beneficial to biomedicine or the production of drugs, chemicals and food. It is for all of these differing motivations or purposes that producers, controllers and researchers rely upon scientific models, by which to explain interactions between a chemical compound (product) and a target organism (which in most cases ultimately is the human being). In the historical times of biomedical research (18th and 19th century), sophisticated instrumentation was not yet available. Back then, it was ingenious to use animals to explore anatomical and physiological features of the human species. Since, animals have been unable to change their status of being the golden standard for any kind of biomedical model, in spite of a dramatic development of other scientific tools, such as all sorts of cell and tissue cultures, computer models, animal-free prescreening tests, sophisticated tools and apparatus for chemical and biological analysis, statistical methods, and well designed tools for clinical testing on humans. No one has the primary aim to afflict pain or ill-being to animals just for the purpose of performing an animal experiment. However, tragically for animals, the accumulated knowledge on biological, physiological and toxicological processes in animals has grown over the decades to an immense mass of information which still remains to be investigated and exploited further. Thus, most resources for the development, production and controlling of products are still based on the animal model approach. Even if certain scientific approaches through animal experimentation and the applicability of their results to humans are not only questioned by animal protectionists but also by scientists, tremendous efforts and time are required to change the direction and points of reference of such an immense industry. Therefore, the initiative and efforts of experts to propagate techniques and approaches in 3R research requires support whenever and wherever possible in order to reduce and replace animal experiments. The statistics on the numbers of animals used in biomedical experiments in 2002 substantiate this demand more convincingly than many words. The present supplement, together with the two former supplements of *ALTEX*, is a contribution towards this objective.

In the first issue (Supplement 1/01) Thomas Hartung and Marlies Halder report on the potential of the three Rs in the area of development and quality control of pharmaceuticals and immunobiologicals.

Thomas Hartung compares *in vitro* to animal tests in the screening of new pharmaceuticals. He attests pharmaceutical companies to be highly willing to incorporate *in vitro* methods into high throughput screening procedures, since animal experiments generally are much more time consuming and resource intensive. Hartung explains that models are most likely to be included in a screening process if they (i) are based on the most recent understanding of the respective disease, (ii) are well characterised to allow interpretation of results, and (iii) require only limited time to perform. He identifies a bottleneck in the technology transfer of new *in vitro* models from academia to industry and concludes that new platforms are necessary to promote this transfer.

Marlies Halder describes the situation in the production and quality control of immunobiologicals, which is regulated by monographs and guidelines issued by international or national pharmacopoeias, international organisations and regulatory bodies. She estimates that approximately 10 million laboratory animals are used worldwide annually to assure the safety and potency of these products. She reports that several animal tests with questionable relevance have been abandoned, and a large number of immunochemical tests have been developed, which have the potential to completely or partly replace the use of animals for potency testing. Halder suggests to seize the opportunity to reduce the number of test animals during the ongoing shift in the quality control concept from reliance on final batch testing to the concept of consistency of production.

In the second issue (Supplement 1/03) a synoptic overview on alternatives to toxicity testing and genetic engineering methodologies is given by Jane Huggins. Brigitte Rusche gives an account on 3R-research and -progress from the point of view of animal welfare.

In her well structured overview article, **Jane Huggins** discusses current trends and issues in the development of alternatives to the use of animals in biomedical experimentation. She considers eight topics, such as the refinement of acute toxicity assays, alternatives to eye and skin corrosion and irritation testing as well as alternatives to reproductive toxicity testing taking into account the state of the art of research, validation activities and the regulatory acceptance of validated *in vitro* alternatives. Huggins also discusses the controversial issue of genetic engineering and transgenic animals as potential means to the reduction of animal use in toxicology. The paper ends with a critical chapter on the process of validation of alternatives to animal testing. Huggins states that validation has just emerged from a rather chaotic phase, in which the principles behind the appropriate performance of a validation study were defined by and by mainly through trial and error.

Brigitte Rusche gives an overview of the entire area of research in the 3Rs through the eyes of an animal welfarist. She evaluates and compares the inherently different positions of an “animal experimentator” and an “animal welfarist” in dealing with 3R-research. The experimentator neither questions the scientific relevance nor ethical acceptability of animal experiments as such. He subordinates his 3R-research activities to the ultimate imperative to protect man from harmful effects of substances and drugs. The “animal welfarist” on the other hand is driven by ethical considerations and puts forward scientific arguments to question the relevance of animal experimentation. Both sides meet when scientists find concrete means to replace animal testing.

In the present issue, Franz Paul Gruber and Thomas Hartung report on alternatives to animal experimentation in basic research, while David Dewhurst and Franz Paul Gruber discuss alternatives in biomedical education and training.

Franz Paul Gruber and **Thomas Hartung** give an overview on the state of the art of animal use and its alternatives in basic bio-

medical research. Since scientists in basic research are free to formulate questions and experimental projects, a large variety of different approaches exist with which to test hypotheses in the broad spectrum of biological and medical disciplines reaching from cancer research and physiology to behavioural research and ecology. Contrary to applied research, e.g. the development of a drug, the only criterion for the choice of a topic in basic research and the ensuing choice of an experimental method, is its scientific relevance. Thus, the authors argue that it is up to the individual scientist to decide what is worth studying and therefore worth using an animal, which also implies ethical considerations.

Gruber and Hartung bring forward an important point of critique concerning the use of animals in basic research: many animal experiments are dramatically "underpowered", i.e. carried out with too few animals to allow conclusions to be drawn from the outcome of the experiments. The authors postulate that a thorough scientifically sound review of the validity of critical animal experiments in basic research should be carried out and made publicly available. The authors list examples of alternatives that were successfully applied in basic research, but, even if published in the scientific literature, have not adopted by other laboratories because they were not well standardised and thus are not reproducible. Due to the crucial role of publishing scientific findings, the authors call for stricter criteria in peer reviewed journals concerning methodological standards and good laboratory practice to help propagate promising methods.

Franz Paul Gruber and **David Dewhurst** present an overview on alternative methods to animal experimentation in biomedical education and training. Although the number of animals used in biomedical training is low in Europe, the need for each animal experiment has to be evaluated carefully, not least because of their exemplary pedagogical role in the training of young future scientists. According to the authors an important problem, which seems to stand in the way of an adequate introduction of alternative methods in this area, is the poor acceptance of alternative methods by some university professors. This problem prevails in spite of positive experiences in countries where the performance of animal experiments is not mandatory for students anymore (Italy, Netherlands) and where the education of biologists, doctors and veterinarians is still good. Typically the most severe problems in replacing animal experiments are found in countries (e.g. the USA, Japan) where the number of animal experiments in education is poorly or not documented at all and where legislation on the protection of animals is less advanced.

What are the lessons to be learned from the six contributions in Supplement 1/2001, Supplement 1/2003 and Supplement 1/2004 of *ALTEX*? Generally speaking, the propagation of 3R alternatives requires further improvement. Depending on the purpose of specific animal experiments, different social mechanisms and official bodies have the power of control over animal experimentation and 3R alternatives. When fighting for alternatives, one has to take these facts into consideration:

1. Biomedical research: Since a good publication record is a prerequisite for the financial support of a research group or single scientist, peer reviewed international scientific journals are, together with funding institutions, in a key position to control the quality and orientation of research projects. They are capable of setting the criteria necessary for an adequate dissemination of 3R techniques. Therefore, journals and funding institutions need to be addressed and convinced.

2. Development of pharmaceutical products: Industries favour screening methods and models, which are based on the most recent understanding of the disease the drug is supposed to cure, which are sufficiently well characterised to allow a reliable interpretation of the data and which require only a minimal development time of the method. These aspects argue in favour of a close collaboration between industry and academia (basic research at universities). Since this collaboration is often jeopardized by the bottle-neck of an insufficient transfer of new screening methods, platforms of technology-transfer need to be developed.

3. Quality control of products in biomedicine: Most manufacturers nowadays produce for the global market. The imperative to eliminate all possible hazards to human health is a prominent cornerstone of the societies in the Western World. The principle that the person or institution that causes damage must bear the costs forces a producer to document that well established and broadly accepted screening and test methods have been applied to discover any hazardous potential of the product. National and international monitoring bodies (e.g. FDA, WHO, OECD, EU, EMEA) and governments decide on the acceptance of such testing methods. New methods need to be validated and compared to established ones. Validation techniques have recently undergone much refinement, including the recognition that validation studies should be built upon a solid platform, consisting of components such as good reference standards, reliable protocol transfer between laboratories, and appropriate application of biostatistical techniques. Thus, validation techniques of 3R alternatives need to be harmonized between countries to enable an optimal worldwide acceptance and use of the data generated with such tests. In addition the willingness of governmental and monitoring bodies to accept validated 3R methods needs to be improved and the time period between a successful validation and the final implementation of the alternative method shortened.

4. Teaching and training: There are sufficient alternative methods to perform good quality teaching in biology, human and veterinary medicine without animal experiments. The trend observed in EU countries to renounce on animal experimentation in basic education and training of students, who do not intend to pursue a scientific career and will never perform animal experiments, should be encouraged and established globally.

We sincerely hope that the three *ALTEX* supplements on the state of the art in alternative methods to animal experimentation help to motivate and inspire scientists at universities and industries, as well as authorities and regulatory boards to develop, promote, evaluate and disseminate methods to reduce, replace and refine tests that are still inflicting pain and distress on animals. We strongly believe that a worldwide implementation of the 3R concept is within the intellectual, moral and technical reach of today's mankind if we give this aim our full attention.

Peter Bossard and Claudia Mertens
President and Member of the Foundation Board FFVFF